thimerosal) as well as a stabilizer (0.2% porcine gelatin) was added to the solution. The resulting mixture was dispensed into appropriate containers, and was used as the hepatitis B vaccine-attenuated cholera toxin injection. The preparation was stored at a temperature of less than 10°C in a cool and dark place.

The hepatitis B vaccine prepared above was inoculated into mice. The antibody production in the blood was evaluated after 3 weeks. The result of passive hemagglutination test showed that the antibody titer was $2^{3.6}$ units in mice subjected to the inoculation of adjuvant-free vaccine and the titer was $2^{5.8}$ units in mice with attenuated cholera toxin.

Example 10 - Pharmaceutical preparation containing vaccine against Japanese encephalitis virus and attenuated cholera toxin (injection):

Japanese encephalitis vaccine was mixed with attenuated cholera toxin (having a toxic activity of at least 1/100000 that of the natural one) that had been dissolved in PBS and sterilized by filtration. 1 ml of the solution contained inactivated Japanese encephalitis virus particles corresponding to $10^{7.0}$ PFU and 2 μg of attenuated cholera toxin. A stabilizer (0.2% porcine gelatin) was added to the solution. The resulting mixture was dispensed into appropriate containers, and was used as the Japanese encephalitis vaccine-attenuated cholera toxin injection. The preparation was stored at a temperature of less than 10°C in a cool and dark place.

The Japanese encephalitis vaccine prepared above was inoculated twice at a 1-week interval into mice. The antibody titer in the blood was evaluated. The titer of neutralizing antibody was $10^{1.8}$ units in mice subjected to the inoculation of the adjuvant-free vaccine, and the titer was $10^{2.9}$ units with attenuated cholera toxin.

Example 11 - Pharmaceutical preparation containing measles-rubella vaccine and attenuated cholera toxin (nasal drop):

Measles-rubella vaccine was mixed with attenuated cholera toxin (having a residual toxic activity of about 1/28500 that of the natural one) dissolved in PBS and sterilized by filtration. 20 μl of the solution contained the respective vaccines of which amounts

corresponded to 7 μg virus particles, and 2.5 μg attenuated cholera toxin. A stabilizer (0.2% porcine gelatin, 0.1% sodium glutamate, 5% lactose) was added to the solution. The resulting mixture was dispensed into appropriate containers, and was used as the nasal drop of measles-rubella vaccine-attenuated cholera toxin. The preparation was stored at a temperature of less than 10°C in a cool and dark place.

The measles-rubella vaccine prepared above was inoculated twice at a 3-week interval into mice. Then the antibody production in the blood was evaluated. The test result showed that the ELISA antibody titer was 0.18 for measles or 0.08 for rubella in mice subjected to the inoculation of the vaccine alone, and the titer was 0.34 for measles or 0.42 for rubella with attenuated cholera toxin-containing vaccine.

Example 12 - Pharmaceutical preparation containing measles-rubella vaccine and attenuated pertussis toxin (nasal drop):

The antibody titer in mouse blood was assayed according to the same method as described in Example 11 except that the attenuated pertussis toxin (the same as prepared in Example 6; having a toxic activity of about 1/100000 that of the natural one when observed by CHO cell morphologic transformation test) was used instead of the attenuated cholera toxin used in Example 11. The ELISA antibody titer was 0.19 for measles or 0.070 for rubella in mice subjected to the inoculation of the adjuvant-free vaccine, and the titer was 0.32 for measles or 0.16 for rubella with attenuated pertussis toxin-containing vaccine.

Example 13 - Pharmaceutical preparation containing rotavirus vaccine and attenuated recombinant *E. coli* heat-labile toxin (oral inoculum, nasal drop):

Rotavirus vaccine was mixed with a fraction of attenuated recombinant LTR(7)K (having a toxic activity of about 1/260000 that of the natural one; the same as in Example 6) that had been dissolved in PBS and sterilized by filtration. 20 μl of the solution contained rotavirus vaccine, of which amount corresponded to 3.5 μg of virus particles, as well as 10 μg attenuated recombinant LTR(7)K. A preservative (0.01% thimerosal) as well as a stabilizer (0.2% porcine

gelatin) was added to the solution. The solution was dispensed into appropriate containers, and was used as the oral inoculum or nasal drop of rotavirus vaccine-attenuated LT toxin. The preparation was stored at a temperature of less than 10°C in a cool and dark place.

The rotavirus vaccine prepared above was inoculated twice at a 3-week interval into mice. Then the antibody titer in the blood was evaluated. The test result showed that the ELISA antibody titer of the intranasal inoculation was 0.072 for the vaccine alone and was 0.40 for attenuated LT toxin-containing vaccine. The titer of oral inoculation was 0.020 for adjuvant-free vaccine and was 0.19 for attenuated LT toxin-containing vaccine.

Industrial Applicability:

Examples described above demonstrate the following:

1. The adjuvant of the invention, comprising attenuated toxin, enhances the production of antibody against coexisting influenza vaccine antigen or the like.
2. Local antibody production, as well as the antibody production in the blood, is enhanced by the adjuvant of the invention when intranasally administered together with vaccine antigen.
3. The adjuvant of the invention exhibits a comparable level of immuno-enhancing activity to that of the natural one at the same dosage, even when the residual toxic activity has been reduced to an undetectable level.
4. The antibody production can be enhanced synergistically in some cases when the adjuvant of the invention is used together with another conventional adjuvant. In other words, the dose of vaccine antigen can be reduced to decrease the incidence of side reaction by using the method in which the inventive adjuvant is used together with another appropriate adjuvant.

Thus, vaccine preparations containing attenuated toxin in accordance with the present invention are useful as adjuvants with high safety. Further, vaccines using the adjuvant of the invention are not only highly safe but also are excellent vaccines having sufficient activities of enhancing immunity even when used by non-injection vaccination route, such as intranasal, percutaneous